ELECTRON MICROSCOPIC STUDY OF HUMAN CORNEA

Somsanguan Ausayakhun, M.D., M.H.Sc.

Department of Ophthalmology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

Abstract
The human cornea was studied by using transmission and scanning electron microscopic techniques. The human corneal epithelium had five to six cell layers which had sparse accumulation of cytoplasmic organelles. Cell-to-cell junctions in the corneal epithelium were desmosomes, gap junctions and tight junctions of the outermost apical cell layer which also had a second membrane specialization, microplicae. The attachment of basal cells to the subjacent stroma was mediated by the adhesion complex, comprising hemidesmosomes, basement membrane, and the anchoring fibrils. Bowman’s layer was an acellular zone, consisting of collagen fibrils densely woven in a random manner into a feltlike matrix. The lamellar stroma was comprised of flattened bundle of collagen fibrils oriented in a parallel manner. Descemet’s membrane had two distinct regions, the anterior fetal layer and the posterior layer which appeared as amorphous matrix. The corneal endothelium was a single layered, low cuboidal cell which contained prominent mitochondria. Studies of this morphological characteristics of normal cornea will be helpful in a better understanding of the pathological processes of various corneal diseases.

Introduction
The cornea functions as a protective membrane and a “window” through which light rays pass to the retina. Maintenance of corneal structure is crucial for its physiologic functions as a biodefense system and as a refractive system. To accomplish these functions the cornea must maintain its strength and transparency. The normally transparent cornea has more recently been altered its curvature by incision or laser to correct the refraction. So it is important to understand the anatomic and morphologic characteristics of the normal cornea in order to better understand its physiologic responses to surgical injuries and other pathology.

Materials and Methods
Corneal specimens were taken from eyes of a 57-year-old Japanese man dying with lymphoma. The tissues were processed according to standard techniques for correlative transmission and scanning electron microscopy.

Results
The human corneal epithelium had five to six cell layers of three different types of epithelial cells: a single layer of columnar basal cells, one to three layers of wing cells, and two to three layers of superficial cells (Fig.1). In all cells, mitochondria and endoplasmic reticulum were sparsely distributed around the ectoplasm and a prominent Golgi apparatus could be seen (Fig.1). The superficial cells were flat, nonkeratinized, polygonal cells which were joined by desmosomes and tight junctions or zonular occludens of the outermost apical cell.
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The cornea consisted of three different cellular layers and two interfaces: epithelium, Bowman’s layer, stroma, Descemet’s membrane, and endothelium. The surface of superficial cells were covered with microvilli, which enlarged the total surface area and allowed the active exchange of oxygen and nutrients between cells and tear fluid. This undulating specialized apical membrane also exhibited a prominent filamentous glycocalyx, which has been studied most extensively in guinea pigs and rats, that had been hypothesized to be associated with the mucus of the tear film layer and to play a role in mucin and tear film spread over the surface eye. Two distinct regions could be detected in electron micrograph: the anterior, fetal or oldest, banded zone (Fig. 5B) and the posterior amorphous, unbanded zone (Fig. 6). The corneal endothelium was a single layered, low cuboidal cell which a large number of mitochondria could be seen in its cytoplasm (Fig. 6).

Discussion

The cornea consisted of three different cellular layers and two interfaces: epithelium, Bowman’s layer, stroma, Descemet’s membrane, and endothelium. The surface of superficial cells were covered with microvilli, which enlarged the total surface area and allowed the active exchange of oxygen and nutrients between cells and tear fluid. This undulating specialized apical membrane also exhibited a prominent filamentous glycocalyx, which has been studied most extensively in guinea pigs and rats, that had been hypothesized to be associated with the mucus of the tear film layer and to play a role in mucin and tear film spread over the surface eye. The number of surface microvilli correlated with the degree of electron scatter, dark cells having the fewest per unit area and being the oldest cell which were about to be desquamated into the tear film.

All cell layers of the epithelium had a rather sparse accumulation of cytoplasmic organelles that perhaps in keeping it to be transparent.

Descemet’s membrane was the basement membrane of the corneal endothelium (Fig. 6). Two distinct regions could be detected in electron micrograph: the anterior, fetal or oldest, banded zone (Fig. 5B) and the posterior amorphous, unbanded zone (Fig. 6).

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Fig. 1. Transmission electron microscope (EM) of human corneal epithelium. A. Five to six layers of epithelial cells overlying a uniform Bowman’s layer (BL) are observed. Note that the cytoplasmic organelles are sparsely distributed with prominent Golgi vesicles (GV). (x 1,500); B. Flattened superficial epithelial cells with numerous microvilli (MP) and numerous desmosomes (arrows) are demonstrated. (x 2,500)

Fig. 2. Transmission EM of apical cells (A) and wing cells (B). A. Apical cells demonstrate microvilli (MP), region of tight junction (TJ), gap junction (GJ) and desmosomes (arrows). (x 8,000) B. Wing cells rich in keratin filaments (KF) and desmosomes (arrows) are observed. (x 15,000)
Anchoring fibrils of type VII collagen were critical to maintain adhesion of basal cells to the basement membrane and stroma. They extended from the basement membrane and reached the stroma where they formed anchoring plaques with type I collagen. A study of the depth of penetration of the anchoring fibril network in diabetic corneas showed a significant decrease in depth compared to age-matched control. These indicated the importance of the anchoring fibril network penetration into the anterior stroma in adhesions of the basement membrane and its epithelium.

Bowman’s layer was considered the anterior portion of the corneal stroma. Based on ultrastructural criteria, embryonically Bowman’s layer was described as being derived from stromal keratocytes, so continuity was observed between collagen fibers in Bowman’s layer and those in the stroma. The function of Bowman’s layer is unclear.

The characteristic feature of the collagen fibers in the corneal stroma was that they were extremely uniform in diameter and the distance between them was also uniform and constant. This regular arrangement of collagen fibers in the stroma contributed to corneal transparency.

The lamellar stroma was secreted and maintained by keratocytes. Keratocytes synthesized a pro-α-chain of collagen, glycosaminoglycans, and also collagen degradative enzymes, such as matrix metalloproteases. Thus, the structural and biochemical homeostasis of the corneal stroma was maintained through cellular regulation of the synthesis and degradation of extracellular matrices.
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Fig. 4A

Fig. 4B

Fig. 5A

Fig. 5B
Fig. 4. Transmission EM of basal cells and Bowman’s layer (BL). A. Basal cells show anterior displacement of nucleus and cell-to-cell junctions of desmosomes (arrows). (x 3,500) B. The epithelial adhesion complex demonstrate hemidesmosomes (HD) and basement membrane (BM). (x 5,000)

Fig. 5. Transmission EM of corneal stroma and anterior part of Descemet’s membrane. A. Lamellar stroma show bundle of collagen fibrils oriented in a parallel manner running in varying directions. Spindle-shaped keratocytes between the lamellae are observed. (x 2,500) B. The anterior part of Descemet’s membrane (DM) show banded zone. (x 6,000)

Fig. 6. Transmission EM of Descemet’s membrane and corneal endothelium. The posterior, unbanded zone of Descemet’s membrane (DM) is observed. The corneal endothelium demonstrates a large number of mitochondria (MT). (x 60,000)

Descemet’s membrane was synthesized by the endothelium and was unique among basement membrane in its thickness and regional variation in structure. Fuchs’ dystrophy were associated with an atypical striated collagen deposition in posterior collagenous layer. Clinically, Descemet’s membrane was tough and resistant to enzymatic degradation by metalloproteases and was tightly adherent to the posterior surface of the corneal stroma and would reflect any change in the shape of the stroma such as when the corneal stroma swelled, the folding of Descemet’s membrane could be observed.
The presence of a large number of mitochondria in the cytoplasm of corneal epithelial cells indicated that they were active in metabolism. The most important physiologic function of corneal endothelium was to regulate the water content of the corneal stroma. Unlike the corneal epithelium, the human corneal endothelium did not replicate and the number of endothelial cells decreased with age. As cells decreased in number, they became thinner and attenuated.

The basal cells of limbal epithelium are hypothesized to be the stem cells for the corneal epithelium. XYZ theory was also proposed as a state of dynamic equilibrium of normal cornea. Thus the limbus, a zone between the cornea and the conjunctiva and the sclera, is an interesting site to be studied further.

Since recently, the normally transparent cornea has been altered by refractive surgery. In order to correct the refractive error, its curvature is modified by incision of or laser interaction with normal corneal tissue. In order to accomplish a better clinical efficacy of refractive surgery, it will be necessary to better understand the structure and function of normal human cornea and its physiological response to surgical injuries.

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References